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EXAMINER	
FOSTER, CHRISTINE E	
ART UNIT	**PAPER NUMBER**
1641	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/620,332	VOYTA ET AL.	
	Examiner	Art Unit	
	Christine Foster	1641	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *15 March 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-45* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-45* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *8/28/03, 3/15/05*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Information Disclosure Statement

1. The Information Disclosure Statements filed August 28, 2003 and March 15, 2005 have been received and have been considered by the examiner except as detailed below.

The U.S. Patent Document by Akhavan-Tafti et al. (reference AB) as listed on Applicant's Information Disclosure Statement filed March 15, 2005 has not been considered as the Document Number (6,668,979) appears to be in error. However, US Patent No. 6,608,979 by Akhavan-Tafti et al. has been cited by the examiner on the attached form PTO-892.

The U.S. Patent Document by Liu et al. (reference AQ) as listed on Applicant's Information Disclosure Statement filed August 28, 2003 has not been considered as the document number does not correspond to a known U.S. Patent Application Publication.

Specification

2. The specification is objected to as failing to provide proper antecedent basis for the claimed subject matter. See 37 CFR 1.75(d)(1) and MPEP § 608.01(o). Correction of the following is required:

Original claim 42 refers to "cyclodextrins." Support for the use of cyclodextrins as additives cannot be found in the specification. Cyclodextrins are also not disclosed in U.S. Patent Nos. 5,145,772 and 5,547,836, or in Copending U.S. Patent Application 10/462,742, which Applicant has incorporated by reference.

Original claims 29-30 refer to "quantifying" the amount of target molecules. The specification does not appear to disclose a method that includes quantifying target molecules.

3. The disclosure is objected to because of the following informalities: The sentence beginning "According to one embodiment" on p. 4, lines 1-2 appears to lack a verb. Appropriate correction is required.

Claim Objections

4. Claim 5 is objected to because of the following informalities: the claim recites a method wherein the "first and second target molecules comprises an antigen moiety." It is suggested that the word "comprises" be replaced with "comprise" for subject-verb agreement.

Claim Rejections - 35 USC § 112

5. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. Claims 10-12 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of detecting chemiluminescent emissions on a solid support wherein probes are disposed on the surface layer at a density of at least 50 or at least 100 discrete areas per cm^2, does not reasonably provide enablement for a method wherein the density is at least 1,000, 25,000 or 50,000

discrete areas per cm^2. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The claims are directed to a method of detecting binding of target species to an array of probes immobilized on a solid support. Binding of targets accompanied by co-immobilization of enzymes capable of cleaving chemiluminescent substrates, which allows for subsequent detection of chemiluminescent emissions when the solid support is contacted with the substrates. A large number of different probes may be immobilized on the support, thereby allowing for screening for the presence of multiple targets in a single assay. The claims are not limited to arrays produced by any particular method and are not limited to a type of probe biomolecule that is immobilized on the support.

Arrays of biomolecules immobilized on solid supports are known in the art and include arrays of proteins (typically known as "protein arrays" or "protein biochips") and nucleic acids ("DNA microarrays" or "cDNA microarrays"). The prior art teaches methods of preparing DNA microrarrays that include ink-jet systems, direct or robotic-aided printing/spotting, and photolithography (Hughes et al., "Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer," *Nature Biotechnology* **19**:342-347 (2001); Reese et al., "Microfabricated Fountain Pens for High-Density DNA Arrays," *Genome Research* **13**:2348-2352 (2003)). Photolithography was successful in creating DNA arrays with approximately 17,000 genes/cm^2 (Reese et al., p. 2348, left column, paragraph 2). Printing using ceramic capillary-tipped pens produced arrays with 10,000 spots/cm^2 (Reese et al., p. 2348, right column, paragraph 1). However, arrays

with feature densities of greater than 25,000 were not commonly known and available at the time of the invention. Moreover, protein-based microarrays were not well known in the art (Joos et al., p. 2641, right column, lines 13-15) and did not approach the feature densities of DNA-based microarrays, with less than 1,000 features (see Mendoza et al., "High-Throughput Microarray-Based Enzyme-Linked Immunosorbent Assay (ELISA)" *BioTechniques* 27:778-788 (1999)).

The prior art teaches detection strategies for use in conjunction with arrays, which include fluorescence and chemiluminescence, with fluorescence detection being most generally used for DNA microarrays (Rajeevan et al., "Chemiluminescent Analysis of Gene Expression on High-density Filter Arrays," *The Journal of Histochemistry* **47**:337-342 (1999), p. 337, left column). The prior art does teach chemiluminescent detection using DNA arrays (e.g., Rajeevan et al; Vernon et al., "Reproducibility of Alternative Probe Synthesis Approaches for Gene expression Profiling with Arrays," *Journal of Molecular Diagnostics* **2**:124-127 (2000); Cheek et al., "Chemiluminescence Detection for Hybridization Assays on the Flow-Thru Chip, a Three-Dimensional Microchannel Biochip," *Anal. Chem.* **73**:5777-5783 (2001)) as well as protein arrays (e.g., Roda et al., "Protein Microdeposition Using a Conventional Ink-Jet Printer," *BioTechniques* **28**:492-496 (2000) (Joos et al., "A microarray enzyme-linked immunosorbent assay for autoimmune diagnostics," *Electrophoresis* **21**:2641-2650 (2000)).

However, in contrast to the feature densities listed above, these prior art teachings of chemiluminescent detection methods employ arrays of more modest

feature densities. Cheek et al. teach 8x8 arrays of probes spotted on 1-cm^2 chips, while the filter arrays of Rajeevan et al. and Vernon et al. comprised 588 spotted targets. Roda et al. teach ink-jet printing of 100-member arrays on a 20 x 20 mm support, with a minimum (Figure 1 and p. 496, middle column). Joos et al. achieved densities on the order of 100 spots per cm^2 (see Joos et al., p. 2643, section 2.2).

Therefore, chemiluminescent detection methods using arrays with densities on the order of 50-100 discrete areas per cm^2 were taught by the prior art at the time of the invention. However, chemiluminescent detection of high-density arrays as claimed in claims 10-12 were not well known in the art, and the specification provides no working examples of any array density.

Regarding production of the probe arrays on the solid support, the specification discloses only that "a plurality of probes are disposed in a plurality of discrete areas on the surface layer" (p. 6) but does not describe or suggest methods of producing high-density arrays or detail how the probes are immobilized on the surface layer according to the instant invention. A number of possible support materials are disclosed, including nitrocellulose, glass, and plastic, but there is no guidance provided regarding how to immobilize probes on the support or how to produce high-density arrays of the claimed densities on these materials. The claims encompass nucleic acid, protein, and other probe types, but the specification further lacks guidance with regard to production of high-density arrays and probe immobilization when the various probe types are to be used according to the invention.

The prior art also teaches that the quality of chemiluminescent signal detection from solid supports is influenced by the type of support used and/or the type of probe immobilized on the support. Roda et al. teach that conventional cellulose paper was a poor support as it gave a poorly localized signal (p. 494, left, column, paragraph 3). Akhavan-Tafti et al. teach that different supports led to substantial variability in apparent spot size, which would affect spatial resolution, and further that certain supports such as glass slides were unsuitable for immobilization of DNA (Akhavan-Tafti et al., "Chemiluminescent Detection of DNA in Low- and Medium-Density Arrays," *Clinical Chemistry* **44**:2065-2066 (1998), p. 2066, right column).

Technical barriers that may be present with chemiluminescent detection and not with fluorescent detection of microarrays include the requirement of enzymes in chemiluminescence detection: Roda et al. teach that chemiluminescent signals may be poorly localized due to washing away of the chemiluminescent enzyme (p. 494, left column, paragraph 3). The specification also discloses that "in contrast to fluorophore-labeled targets, the use of enzyme labeled targets and chemiluminescent substrates results in a signaling species...which is not attached to the support and which is therefore free to migrate during the assay...[which]...can reduce the spatial resolution of the assay (see p. 2, lines 10-20). The specification discloses flash kinetics (p. 4, lines 18-23), which is also taught by Cheek et al. to address the problem of spatial resolution (p. 5779, left column, second paragraph). However, the specification lacks guidance regarding how to use the methods of the invention with the substantially higher density microarrays claimed in claims 10-12.

Due to the state of the prior art, which establishes that microarrays of the claimed densities, and in particular chemiluminescence detection in conjunction with such microarrays, were not well known in the art at the time of the invention, the lack of direction/guidance presented in the specification regarding production of such high-density arrays and chemiluminescence detection methods employing them, the absence of working examples directed to same, the unpredictability of adequate signal detection using various support materials, and the breadth of the claims, which encompass both protein, DNA, and other arrays produced by any method, the specification fails to teach the skilled artisan how to make and use the claimed invention without undue experimentation.

7. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

8. Claims 1-45 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

9. Claim 1 recites "detecting first chemiluminescent signal" and "detecting second chemiluminescent signal" (lines 7-8 and 12-13 of the claim). It is unclear whether the first and second chemiluminescent signals that are being detected are those recited previously in the claim (lines 6 and 11). It is also suggested that the claim be amended to insert an article such as "the" or "a" between the words "detecting" and "first."

Claim 1 recites a plurality of probes "disposed in" a plurality of discrete areas. The specification also discloses probes "disposed in" a plurality of discrete areas on the surface layer of a solid support (p. 6, lines 11-12). The use of the term "disposed in" renders the claim indefinite. For example, it is not clear whether the probes are immobilized on the surface of the solid support (e.g. by physical adsorption).

Claim 1 is rejected as vague and indefinite for recitation of probes, "wherein at least some of the probes are bound to a first enzyme conjugate...and at least some of the probes are bound to a second enzyme conjugate." It is unclear whether the probes are bound to the enzyme conjugates throughout the method (e.g., covalently bound), or whether the probes become bound to the enzyme conjugates at some point in time during the performance of one of the method steps.

10. Claims 3-4 and 7 recite methods according to claim 1, wherein enzyme conjugates are bound to a probe. The claims are indefinite because it is unclear whether the probes recited in claims 3-4 and 7 are one of the "plurality of probes" recited in claim 1, or a distinct probe.

11. Claims 3-4 and 7 recite methods according to claim 1, wherein enzyme conjugates are "bound to" a probe. Claim 4 additionally recites target molecules that are "bound to" probes. The claims are indefinite because it is unclear whether the enzyme conjugates and target molecules are bound to the probes throughout the method (e.g., covalently bound), or whether the enzyme conjugates and target molecules become bound to the enzyme conjugates at some point in time during the performance of one of the method steps.

12. Claim 5 recites "the antibody" in line 3. There is insufficient antecedent basis for this limitation as there is no prior mention of an antibody in the claim.

13. Claim 8 recites the limitation "the chemiluminescent quantum yield enhancing material". There is insufficient antecedent basis for this limitation. Claim 2 refers to a *composition* comprising such a material.

14. Claims 13, 21, 22, 24, 26, 30, and 37 recite the limitation "the support surface." There is insufficient antecedent basis for this limitation in the claims.

15. Claim 13 recites the limitation "the substrate composition" (line 4). There is insufficient antecedent basis for this limitation in the claim.

16. Claim 13 recites the limitation "the substrate composition" (line 4), which renders the claim indefinite as there are two substrate compositions recited in claim 1.

17. Claim 15 is rejected as vague and indefinite for recitation of a "moiety." It is unclear what is meant by this term.

18. Claim 17 is rejected as vague and indefinite for recitation of mRNA transcripts "derived from" mRNA transcripts. It is unclear in what sense the mRNA transcripts are derived from mRNA transcripts. It is further unclear if the mRNA transcripts are identical to those they are derived from.

19. Claim 18 is rejected as vague and indefinite for recitation of cDNA or cRNA transcripts "derived from" mRNA transcripts. It is unclear in what sense the transcripts are derived from mRNA transcripts.

20. Claim 23 recites the limitation "the analyte probes" in line 2. There is insufficient antecedent basis for this limitation in the claim.

21. Claim 24 is rejected as vague and indefinite as it recites a support surface that "further comprises fluorescent labels." It is unclear in what sense the support surface comprises fluorescent labels; for example, whether they are attached to the surface or are attached to probes on the support surface.

22. Claim 25 is rejected as vague and indefinite as it recites chemiluminescent signals that have "different emission maxima." The specification discloses that differences in emissions of chemiluminescent signals with different emission maxima are detectable and can emit different colors (p. 13, lines 7-12), but does not specify what constitutes "different" emission maxima.

23. Claim 30 recites the limitation "the signal" in line 3. There is insufficient antecedent basis for this limitation in the claim.

24. Claim 30 is rejected as vague and indefinite as it recites a support surface that "further comprises fluorescent labels." It is unclear in what sense the support surface comprises fluorescent labels; for example, whether they are attached to the surface or are attached to probes on the support surface.

25. Claim 31 recites a method step performed "after incubating." There is insufficient antecedent basis for this limitation in the claim, as there is no prior mention of an incubating step.

26. Claim 38 recites "the antibody" in line 3. There is insufficient antecedent basis for this limitation as there is no prior mention of an antibody in the claim.

Claim 38 also recites "an antigen for the antibody." The claim is indefinite because the relationship between antigen and antibody is not clear.

27. Claims 35-36 recite a composition comprising a chemiluminescent substrate that is a 0.1M solution of buffer and 1 mM $MgCl_2$. The use of the word "is" suggests that the composition consists only of the recited ingredients, but lines 1-2 of the claims indicate that the composition also comprises a chemiluminescent substrate.

Claim Rejections - 35 USC § 102

28. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

29. Claims 1, 3-4, 13-15, 21, 29, and 31-32 are rejected under 35 U.S.C. 102(b) as being anticipated by Cheek et al. ("Chemiluminescence Detection for Hybridization Assays on the Flow-Thru Chip, a Three-Dimensional Microchannel Biochip," *Anal. Chem.* **73**:5777-5783 (2001)).

Cheek et al. teach a method of sequentially detecting chemiluminescent emissions on a solid support (microchannel glass) that includes the steps of contacting a surface layer of the solid support with a composition comprising a first chemiluminescent substrate (the luminol-based substrate Super Signal West Femto Maximum Sensitivity Substrate) capable of being activated by a first enzyme (horseradish peroxidase) to produce a first chemiluminescent signal; detecting the first chemiluminescent signal (see p. 5780, left column, "Detection," and pp. 5781-5782, "Two-Channel Chemiluminescence"); contacting the surface layer of the solid support

with a composition comprising a second chemiluminescent substrate (the acridan phosphate-based substrate APS-5) capable of being activated by a second enzyme (alkaline phosphatase) to produce a second chemiluminescent signal; detecting the second chemiluminescent signal; wherein a plurality of probes are disposed in a plurality of discrete areas on the surface layer at a density of 64 discrete areas per cm^2 (see p. 5778, lines 2-14; p. 5779, left column, "Chip Preparation," lines 1-4 and right column, lines 11-12).

The first and second enzyme conjugates are each bound indirectly to a probe (biotin-labeled target molecules are bound to streptavidin-HRP conjugates via biotin-streptavidin interaction and FITC-labeled targets are bound to anti-fluorescein-alkaline phosphatase conjugates via FITC/anti-fluorescein interaction). The labeled nucleic acids are contacted with the support surface prior to addition of the substrate composition (see Table 1 and pp. 5781-5782, "Two-Channel Chemiluminescence"). Detection and quantification of target molecule binding to discrete areas of the chip is through image analysis (p. 5782, left column and Figure 5). The solid support is washed before contact with the first and second substrate compositions (p. 5779, "Hybridization assay," and p. 5782, left column).

30. Claims 25 and 27 are rejected under 35 U.S.C. 102(b) as being anticipated by Cheek et al. as evidenced by Weimer et al. (US Patent Application Publication 2004/0009529 A1). Cheek et al. is as discussed above, which fails to specifically recite that the luminol and acridan phosphate-based substrates employed have the same or different emission maxima.

Weimer et al. teach that luminol emits light at a maximum of 425 nm, and that APS-5 emits light at a maximum of 430 nm (paragraph 66). Therefore, the luminol and acridan phosphate-based substrates of Cheek et al. inherently anticipate claim 25, as they have different emission maxima. With respect to claim 27, because the instant specification and claims do not define what wavelengths or range of wavelengths constitute "approximately the same" emission maxima, the examiner has considered that the emission maxima of 425 and 430 nm could fulfill this limitation as well, thereby anticipating instant claim 27.

Claim Rejections - 35 USC § 103

31. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

32. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

33. Claims 2, 5, 28, and 44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. (*Anal. Chem.* **73**:5777-5783 (2001)) in view of Akhavan-Tafti (US Patent No. 6,068,979).

Cheek et al. is as discussed above, which fails to fails to specifically recite that the chemiluminescent substrate compositions are contacted with the support in the presence of a quantum yield enhancing material. With regard to claim 44, Cheek et al. fails to teach antidigoxigenin:enzyme conjugates wherein the corresponding target molecules are labeled with digoxigenin. Cheek et al. also fail to specifically recite that the compositions comprising the chemiluminescent substrates are buffered compositions.

Akhavan-Tafti '979 teaches a method for sequential detection of multiple analytes by chemiluminescent emission on a solid support (the abstract), wherein surfactant enhancers are used to improve the signal/background ratio of enzymatically produced chemiluminescence. Akhavan-Tafti '979 further teaches that suitable surfactant enhancers are known in the art and include polymeric onium salts, including quaternary phosphonium salts and ammonium salts, monomeric quaternary phosphonium and ammonium salts such as cetyltrimethylammonium bromide and dicationic surfactants (column 10, lines 29-47).

Akhavan-Tafti '979 teaches two chemiluminescent substrates that are employed in the method and are used in buffered compositions (column 10, lines 42-47 and column 15, lines 1-17). Akhavan-Tafti further teach that the dioxetane chemiluminescent

substrate LUMIGEN PPD is commercially available as the composition LUMI-PHOS PLUS, which comprises an alkaline buffer solution.

Akhavan-Tafti '979 teaches binding pairs, including antigen-antibody and biotin-avidin or streptavidin. One member of a binding pair may be attached to an enzyme form an enzyme conjugate, which is then capable of interacting with a target molecule labeled with the other member of the binding pair (column 4, lines 30-40 and column 5, lines 18-28). Specific examples of antigen-antibody binding pairs include antigoxigenin-digoxigenin, and antidigoxigenin:enzyme conjugates are disclosed (columns 15-16, Example 2).

Therefore, it would have been obvious to one of ordinary skill in the art to employ surfactant enhancers, as taught by Akhavan-Tafti '979, in order to improve the chemiluminescent signal/background ratio in a method for sequential detection of enzymatically produced chemiluminescence, such as that of Cheek et al. It would also have been obvious to one of ordinary skill in the art to employ buffered compositions comprising chemiluminescent substrates in the method of Cheek et al. because Akhavan-Tafti teaches that such compositions are commercially available in that form and are useful in a method for sequential detection of chemiluminescent emissions on a solid support, such as that of Cheek et al.

It would also have been obvious to one of ordinary skill in the art at the time of the invention to substitute the streptavidin-HRP conjugate of Cheek et al. with an antidigoxigenin-HRP conjugate for detection of a second target molecule labeled with digoxigenin, because Akhavan-Tafti '979 teaches that antigen-antibody and biotin-

streptavidin are both examples of binding pairs that may be successfully used in a method of sequential chemiluminescent detection of multiple, differentially labeled target molecules such as that of Cheek et al.

34. Claims 1-5, 7, 9, 13-15, 20-21, 28-29, 31-32, 40-41, and 44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti (US Patent No. 6,068,979) in view of Akhavan-Tafti et al. ("Chemiluminescent Detection of DNA in Low- and Medium-Density Arrays," *Clinical Chemistry* **44**:2065-2066 (1998)).

Akhavan-Tafti '979 teaches a method of detecting chemiluminescent emissions on a solid support substantially as claimed. The method includes the steps of contacting a surface layer of the solid support with a composition comprising a first chemiluminescent substrate capable of being activated by a first enzyme to produce a first chemiluminescent signal, detecting the first chemiluminescent signal on the surface layer of the solid support, contacting the surface layer of the solid support with a composition comprising a second chemiluminescent substrate capable of being activated by a second enzyme to produce a second chemiluminescent signal, and detecting the second chemiluminescent signal on the surface layer of the solid support (see in particular column 6, lines 17-24, 41-45 and 60-67; and column 7, lines 1-46; column 10, lines 59-67; and column 11, lines 1-44).

Akhavan-Tafti '979 also discloses use of chemiluminescent quantum yield enhancing materials, which may be onium copolymers (column 10, lines 24-47) including poly(vinylbenzylammonium salts) and which may be present in the chemiluminescent substrate composition (see column 9, lines 46-52; column 10, lines

15-47 and US Patent 5,45,347, which was incorporated by reference). Chemiluminescent substrates are employed as buffered compositions (column 9, lines 46-47; column 10, lines 42-45, and column 15, lines 1-8). Washing of the solid support may be performed prior to contacting with the first substrate composition (column 15, lines 48-51) or after the first detection step (column 13, lines 9-13).

Akhavan-Tafti '979 teaches first and second enzyme conjugates that are bound directly to probes or are bound to first and second target molecules that are bound to probes (columns 15-16, Example 2; column 14, lines 29-33 and 60-67; column 4, lines 31-40; column 5, lines 18-24). Also disclosed are antidigoxigenin:enzyme conjugates wherein the corresponding target molecules are labeled with digoxigenin ((columns 15-16, Example 2).

Akhavan-Tafti '979 also discloses contacting a support surface with a sample comprising first and second target molecules labeled with a first second label (e.g., lambda phage DNA labeled with biotin and SPPI marker DNA labeled with digoxigenin) prior to contacting the support surface with the substrate composition (columns 15-16, Example 2 in particular). Target molecules can include pools of target nucleic acids and mRNA for expression studies (column 14, lines 12-17) and may be quantified (column 1, lines 55-58).

Akhavan-Tafti '979 fails to specifically teach a method wherein a plurality of probes is disposed on the surface layer at a density of at least 50 or at least 100 discrete areas per cm^2.

However, Akhavan-Tafti et al. teach chemiluminescent detection of DNA in low- and medium-density arrays of 100 spots per cm^2 (p. 2065, right column, paragraph 4). Akhavan-Tafti further teach that such arrays are useful in high-throughput analysis of gene mutations and gene expression (p. 2065, right column, paragraph 1) and can be combined with chemiluminescent analysis with no expensive instrumentation (p. 2066, right column, last paragraph).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to include the plurality of probes disposed on a surface layer at a density of at least 50 or 100 discrete areas per cm^2 as taught by Akhavan-Tafti et al. in the method of detecting chemiluminescent emissions of Akhavan-Tafti '979, because Akhavan-Tafti et al. teaches the benefit of arrays in allowing for high-throughput analysis in methods for chemiluminescent detection of biological molecules, such as that of Akhavan-Tafti '979. One would have had reasonable expectation of success in combining the array of Akhavan-Tafti et al. with the sequential chemiluminescent detection method of Akhavan-Tafti '979 because Akhavan-Tafti et al. established that chemiluminescent detection was feasible with array formats.

35. Claim 45 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti (US Patent No. 6,068,979) as applied to claims 2, 5, 38, and 44 above, or, alternatively, Akhavan-Tafti '979 in view of Akhavan-Tafti et al., and further in view of Wang et al. (WO 01/73134 A2).

As discussed above, Akhavan-Tafti '979 teaches binding pairs such as digoxigenin-antidigoxigenin in methods of determining chemiluminescent emissions on

a solid support. Akhavan-Tafti '979 also teaches sequential chemiluminescent detection for measuring levels of target species such as mRNA (column 6, lines 25-34). However, Cheek et al. and Akhavan-Tafti '979 et al. fail to teach a method wherein cDNA target molecules are labeled with digoxigenin.

Wang et al. teach ordered arrays of pools of target molecules (nucleic acids) on a solid support, where the mixtures reflect the expression profile of different cells or tissues (the abstract). Target molecules include cDNA (p. 24, lines 28-32 and p. 26, lines 24-33), which can be used in microarray methods for analysis of gene expression in place of mRNA target samples because cDNA is more stable.

It would have been further obvious to one of ordinary skill in the art to employ cDNA target molecules as taught by Wang et al. in the method of sequential chemiluminescent detection using digoxigenin-antidigoxigenin binding of Cheek et al. and Akhavan-Tafti '979 when detecting mRNA target species, because Wang teaches that cDNA target molecules derived from mRNA are more stable. One would have reasonable expectation of success because Wang further teaches that cDNA target molecules may be effectively used in array formats, such as that of Cheek et al. and Akhavan-Tafti '979.

36. Claim 7 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Huang ("Detection of multiple proteins in an antibody-based protein microarray system," *Journal of Immunological Methods* **255**:1-13 (2001)).

Cheek et al. fail to teach a method wherein the first and second enzyme conjugates are each bound directly to probes; that is, where the target molecules in the sample are directly labeled with an enzyme (see the specification, p. 7, lines 22-24).

Huang teaches detection of multiple proteins in a protein microarray using enhanced chemiluminescence detection. In one embodiment, immunoglobulin probes were spotted onto a membrane and detected by incubation with antibody targets that were conjugated with HRP (see p. 4-6, section 3.1 and Table 2). Huang teaches that detection of the HRP targets binding to immobilized immunoglobulins was useful in testing the specificity and sensitivity of the assay (p. 6, left column, paragraphs 2-4).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ target molecules directly labeled with an enzyme, as taught by Huang et al., in order to assess the specificity and sensitivity of an assay involving a protein microarray with chemiluminescent detection, such as that of Cheek et al.

37. Claims 8 and 40-42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti (US Patent No. 6,068,979) as applied to claims 2, 5, 28, and 44 above, and further in view of Bronstein et al. (US Patent No. 6,602,658 B1).

Cheek et al. and Akhavan-Tafti '979 are as discussed above, which fail to teach a method wherein the surface layer is contacted with quantum yield enhancing material before contacting the surface layer with the composition comprising the first chemiluminescent substrate, or where the quantum yield enhancing material is an

onium polymer selected from the group listed in claim 40, an onium copolymer, or where the composition comprising the quantum yield enhancing material further comprises an additive selected from the group listed in claim 42.

Bronstein et al. teach a method of measuring gene activity using sequential chemiluminescent detection of signal from two or more chemiluminescent substrates, wherein chemiluminescent signal enhancers such as polyvinylbenzyltrimethylammonium chloride (TMQ), onium copolymers, or BSA may be added to increase the intensity of the chemiluminescent signals in aqueous medium (column 11, lines 29-61). Bronstein et al. further teach that the enhancer molecule can be added at any point during the method (column 13, lines 39-52).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to provide the quantum yield enhancing material before contacting the surface layer with the first chemiluminescent substrate composition because Bronstein et al. teaches that the enhancer may be successfully added at any point in a methods for chemiluminescent detection of multiple substrates, such as those of Cheek et al. and Akhavan-Tafti '979. It would have been further obvious to include BSA as an additive because Bronstein et al. teach that both BSA and onium polymers serve to enhance the chemiluminescent signal. In addition, it would have been obvious to employ polyvinylbenzyltrimethylammonium chloride or an onium copolymer because Bronstein et al. teach that these are enhancers capable of significantly increasing the intensity of the chemiluminescent signal emitted in chemiluminescent detection methods.

38. Claim 20 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Bronstein et al. (US Patent No. 6,602,658 B1). Cheek et al. and Bronstein et al. are as discussed above, which fail to teach a method wherein the first and second chemiluminescent substrates are each contacted with the surface layer in the presence of a chemiluminescent quantum yield enhancing material.

As discussed above, Bronstein et al. teach a method wherein enhancers such as onium copolymers, polyvinylbenzyltrimethylammonium chloride or BSA may be added to increase the intensity of the chemiluminescent signals in aqueous medium (column 11, lines 29-61). Bronstein et al. further teach that the enhancer molecule can be added at any point during the method (column 13, lines 39-52).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to contact the chemiluminescent substrates with the surface layer in the presence of a chemiluminescent quantum yield enhancing material because Bronstein et al. teach that the chemiluminescent enhancers serve to increase the intensity of the chemiluminescent signals, and that the enhancing material may be added at any point in a method of detection of chemiluminescent signals such as that of Cheek et al.

39. Claim 9 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti et al. (*Clinical Chemistry* **44**:2065-2066 (1998)).

Cheek et al. fail to teach a method wherein the density of discrete areas on the surface layer is at least 100 discrete areas per cm^2.

As discussed above, Akhavan-Tafti et al. teach chemiluminescent detection of DNA in low- and medium-density arrays of 100 spots per cm^2 (p. 2065, right column, paragraph 4). Akhavan-Tafti et al. further teach that such arrays are useful in high-throughput analysis of gene mutations and gene expression (p. 2065, right column, paragraph 1) and can be combined with chemiluminescent analysis with no expensive instrumentation (p. 2066, right column, last paragraph).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to include the plurality of probes disposed on a surface layer at a density of at least 50 or 100 discrete areas per cm^2 as taught by Akhavan-Tafti et al. in the method of detecting chemiluminescent emissions of Cheek et al., because Akhavan-Tafti et al. teaches the benefit of such higher density arrays in allowing for high-throughput analysis in methods for chemiluminescent detection of biological molecules, such as that of Cheek et al.

40. Claims 16-19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. or, alternatively, Akhavan-Tafti '979 in view of Akhavan-Tafti et al., and further in view of Wang et al. (WO 01/73134 A2).

Akhavan-Tafti '979, Akhavan-Tafti et al., and Cheek et al. are as discussed above, which fail to teach methods wherein the target molecules are pools of nucleic acids, comprise mRNA transcripts, cDNA or cRNA transcripts, or wherein the concentration of target nucleic acids is proportional to the expression level of genes.

Wang et al. teach ordered arrays of pools of target molecules (nucleic acids) on a solid support, where the mixtures reflect the expression profile of different cells or

tissues (the abstract). Detection by nucleic acid hybridization using labeled probes may be through chemiluminescence (p. 3, line 35 to p. 4, line 2). Wang et al. further teach that target molecules may comprise mRNA or may be DNA derived from mRNA (such as cDNA) in order to provide a relatively accurate indication of the level of expression of each gene in a cell (see p. 24, lines 21-37 and p. 26, lines 24-28 in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ the chemiluminescence detection method of Cheek et al. or Akhavan-Tafti '979 and Akhavan-Tafti et al. to detect pools of nucleic acids such as mRNA or cDNA as taught by Wang et al. because Wang et al. teach that such mRNA or mRNA-derived target molecules indicate of the level of expression of each gene in a cell, making them useful for gene expression studies. One would have reasonable expectation of success in using these target molecules in the methods of Cheek et al. and Akhavan-Tafti '979/Akhavan-Tafti et al. because Wang et al. teach that chemiluminescent detection may be employed in a nucleic acid hybridization detection methods.

41. Claims 22-23 and 30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. or, alternatively, Akhavan-Tafti '979 view Akhavan-Tafti et al., in further in view of Ferea et al. (US Patent 6,905,826 B2). Cheek et al., Akhavan-Tafti '979 and Akhavan-Tafti et al. fail to teach a method wherein control probes are located on the support surface, or wherein control probes are co-located in one or more of the same discrete areas as the analyte probes.

Ferea et al. teach methods for detecting of target molecules in a sample using nucleic acid microarrays and in particular controls signals to be used in such methods. Such control signals allow for correction of irregularities in the shape, size, and intensity of microarray features (column 5, lines 49-52). Control signals additionally may be used to quantify the experimental signal (column 6, lines 16-19). Control oligonucleotide probes deposited on the array in the same discrete areas ("features") as the experimental probes can be used as hybridization controls (see column 6, lines 41-60; claim 1 and Figure 4 in particular). Control labels may include fluorescent labels, to be used in conjunction with chemiluminescence labeling of experimental target molecules (see claim 17 in particular).

Ferea et al. further teach introduction of control labels, which may include fluorescent labels, to the surface of the array, which are used to calculate the relative amount of multiple experimental target sequences by comparing the ratios of the intensity of the experimental and control label signals (column 6, lines 15-19 and claim 15).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to include the control probes taught by Ferea et al. in the method of Cheek et al. or Akhavan-Tafti '979 and Akhavan-Tafti et al. because Ferea et al. teach the benefit of control probes in determining whether hybridization is occurring in a microarray-format nucleic acid hybridization method.

It would have been further obvious to include in the method of Cheek et al. or Akhavan-Tafti '979 and Akhavan-Tafti et al. a fluorescent label as a control label and to

compare the intensity of the signal from the fluorescent label to the experimental chemiluminescent signals because Ferea et al. teach that such control labels may be used to help quantify experimental signals in a microarray-format nucleic acid hybridization method using chemiluminescent and/or fluorescent detection.

42. Claim 24 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Yang et al. (WO 01/83814 A2). Cheek et al. fail to teach a method wherein the support surface further comprises fluorescent labels.

Yang et al. teach a method for analysis of gene expression, wherein probes are immobilized on a solid support (bead) containing a fluorochrome (see the abstract and p. 15, lines 4-9 and 14-15 and p. 17, lines 12-23). Detection of hybridization of a target nucleic acid molecule to the immobilized probe may be through chemiluminescence (p. 3, lines 4-5 and p. 17, lines 3-11).

43. Claim 26 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. or, alternatively, Akhavan-Tafti '979 in view of Akhavan-Tafti et al., and further in view of Gambini et al. (US Patent No. 6,518,068 B1). Cheek et al., Akhavan-Tafti '979 and Akhavan-Tafti et al. fail to teach a method wherein detection of the second chemiluminescent signal comprises filtering the emissions with a filter adapted to reduce the intensity of the first chemiluminescent signal relative to the intensity of the second.

Gambini et al. teach a detection workstation for analysis of luminescent signals that comprises a filter (or filters on a filter wheel) which permits the selection of different wavelength ranges, and which may be used to separate the emissions of different

reagents emitting at different wavelengths. The workstation may be used in a method for detecting multiple luminescent signals emitting at different wavelengths (see the abstract and column 6, line 55 to column 7, line 13). Gambini et al. further teach that signals from multiple reagents are separated using the filters, which are designed to maximize the sensitivity of the target reagent emission, while minimizing the sensitivity to other non-target reagent emission (column 13, line 60 to column 14, line 10).

Therefore, it would have been obvious to one of ordinary skill in the art to employ the filter detection method taught by Gambini et al. in the methods of Cheek et al. or Akhavan-Tafti '979 and Akhavan-Tafti et al. because Gambini et al. teach that such filters may be used to separate signals at different wavelengths by multiple reagents in a method for detection of multiple luminescent signals.

44. Claim 34 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Bronstein et al. (US Patent No. 4,931,223). Cheek et al. fail to teach a method wherein the two enzymes are beta-galactosidase and alkaline phosphatase.

Bronstein et al. teach methods of chemiluminescent detection for detection of multiple analytes in a sample, employing two enzyme conjugates that are beta-galactosidase and alkaline phosphatase conjugates (column 8, lines 1-21).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to substitute beta-galactosidase as taught by Bronstein et al. for the horseradish peroxidase enzyme used by Cheek et al. because Bronstein et al. teach that beta-galactosidase is also a suitable enzyme for use in conjunction with alkaline

phosphates in a two-enzyme chemiluminescence method for detection of multiple analytes in a sample, such as that of Cheek et al.

45. Claim 35 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Bronstein et al. as applied to claim 34 above, or, alternatively, Akhavan-Tafti '979 in view of Akhavan-Tafti et al. as applied to claims 1-5, 7, 9, 13-15, 20-21, 28-29, 31-32, 40-41, and 44, and further in view of Voyta et al. (US Patent No. 5,145,772). Cheek et al. and Bronstein et al. fail to teach a method wherein the composition comprising a chemiluminescent substrate capable of being activated by alkaline phosphatase is a 0.1M solution of aminomethylpropanol and 1 mM $MgCl_2$ at a pH of 9.5.

Voyta et al. teach a chemiluminescence detection method wherein alkaline phosphatase is used in a solution containing 0.05M carbonate or Tris buffer solution and 1 mM $MgCl_2$ at pH=9.5 (column 11, lines 49-55 and column 13, lines 10-17). Although Voyta et al. teach carbonate rather than sodium phosphate, it is well known in the art that these buffers may be interchanged in order to maintain a solution pH of 9.5. Further, while the concentration of the buffer used by Voyta et al. differs, such a difference will generally not support patentability in most cases, constituting optimization of ranges within prior art conditions (see MPEP 2144.05).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ a solution comprising a buffer with a buffering capacity sufficient to maintain a pH of 9.5 in conjunction with 1 mM $MgCl_2$ in the method of Cheek et al. and Bronstein et al. because Voyta teaches that such a solution is effective in a chemiluminescence detection method employing alkaline phosphatase.

46. Claim 36 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view Bronstein et al., or, alternatively, Akhavan-Tafti '979 in view of Akhavan-Tafti et al., and further in view of Bobrow et al. (US Patent No. 5,196,306). Cheek et al. and Bronstein et al. fail to teach a method wherein the composition comprising a chemiluminescent substrate capable of being activated by beta-galactosidase is a 0.1M solution of sodium phosphate and 1 mM $MgCl_2$ at a pH of 7.0.

Bobrow et al. teach use of beta-galactosidase in a solution comprising of 10 mM sodium phosphate, 1 mM $MgCl_2$ at pH 7.0 (column 17, paragraph 2). Beta-galactosidase stored in this solution was shown to be active (Figure 7).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ a substrate solution containing 0.1M solution of sodium phosphate and 1 mM $MgCl_2$ at a pH of 7.0 because Bobrow et al. teach that a solution comprising sodium phosphate buffer and 1 mM $MgCl_2$ was an appropriate solution that would not destroy enzyme activity in an assay involving beta-galactosidase, such as that of Cheek et al. and Bronstein et al.

47. Claims 37-38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti '979 in view of Akhavan-Tafti et al. as applied to claims 1-5, 7, 9, 13-15, 20-21, 28-29, 31-32, 40-41, and 44 above, and further in view of Clothier (US Patent No. 6,852,503 B1). Akhavan-Tafti '979 in view of Akhavan-Tafti et al. fail to specifically recite contacting a support surface with a composition comprising the first and second enzyme conjugates. However, Akhavan-Tafti '979 teaches binding pairs, including antigen-antibody and biotin-avidin or streptavidin. One member of a binding pair may be

attached to an enzyme form an enzyme conjugate, which is then capable of interacting with a target molecule labeled with the other member of the binding pair (column 4, lines 30-40 and column 5, lines 18-28). Specific examples of antigen-antibody binding pairs include antigoxigenin-digoxigenin, and antidigoxigenin:enzyme conjugates are disclosed (columns 15-16, Example 2).

Clothier teaches a dual enzyme chemiluminescent substrate formulation for use in methods involving two enzymes. Clothier teaches combining the two chemiluminescent enzymes (horseradish peroxidase and alkaline phosphatase) together prior to contacting the enzymes with the well surface (column 6, lines 25-36).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the enzyme conjugates of Akhavan-Tafti '979 prior to contacting with the support surface since Clothier teaches that enzymes for chemiluminescent substrates may be successfully combined together in methods for chemiluminescence detection involving two enzymes, such as that of Akhavan-Tafti '979. It would have been further obvious to employ this step taught by Clothier in a method wherein the first and second enzyme conjugates that comprise enzyme-antibody conjugates and wherein the first and second target molecules are labeled with an antigen for the antibody because antibody-enzyme conjugates are taught by Akhavan-Tafti '979.

48. Claim 37 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. as applied to claims 1, 3-4, 13-15, 21, 29 and 31-32 above, and further in view of Clothier (US Patent No. 6,852,503 B1). Cheek et al. fail to specifically recite contacting

a support surface with a composition comprising the first and second enzyme conjugates.

Clothier teaches a dual enzyme chemiluminescent substrate formulation for use in methods involving two enzymes. Clothier teaches combining the two chemiluminescent enzymes (horseradish peroxidase and alkaline phosphatase) together prior to contacting the enzymes with the well surface (column 6, lines 25-36).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the enzyme conjugates of Cheek et al. and Akhavan-Tafti '979 prior to contacting with the support surface since Clothier teaches that enzymes for chemiluminescent substrates may be successfully combined together in methods for chemiluminescence detection involving two enzymes, such as that of Cheek et al. and Akhavan-Tafti '979.

49. Claim 38 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Clothier as applied to claim 37 above, and further in view of Akhavan-Tafti '979. Cheek et al. and Clothier et al. fail to teach a method wherein both the first and second enzyme conjugates that comprise enzyme-antibody conjugates and wherein the first and second target molecules are labeled with an antigen for the antibody

Akhavan-Tafti '979 teaches binding pairs, including antigen-antibody and biotin-avidin or streptavidin. One member of a binding pair may be attached to an enzyme form an enzyme conjugate, which is then capable of interacting with a target molecule labeled with the other member of the binding pair (column 4, lines 30-40 and column 5,

lines 18-28). Specific examples of antigen-antibody binding pairs include antigoxigenin-digoxigenin, and antidigoxigenin:enzyme conjugates are disclosed (columns 15-16, Example 2).

Therefore, it would also have been obvious to one of ordinary skill in the art at the time of the invention to substitute the streptavidin-HRP conjugate of Cheek et al. with an antidigoxigenin-HRP conjugate for detection of a second target molecule labeled with digoxigenin, because Akhavan-Tafti '979 teaches that antigen-antibody and biotin-streptavidin are both examples of binding pairs that may be successfully used in a method of sequential chemiluminescent detection of multiple, differentially labeled target molecules such as that of Cheek et al.

50. Claim 39 is rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti '979 in view of Akhavan-Tafti et al. as applied to claims 1-5, 7, 9, 13-15, 20-21, 28-29, 31-32, 40-41, and 44 above, and further in view of Greene et al. (US Patent No. 5,137,804).

Akhavan-Tafti '979 and Akhavan-Tafti et al. are as discussed above, which fail to teach a method wherein the first substrate is a 1,2-dioxetane substrate and the second is an acridan, enol phosphate, or luminol substrate. Akhavan-Tafti '979 teaches that the first chemiluminescent substrate used in a sequential detection method must be capable of being inhibited, and that the use of a horseradish peroxidase substrate such as an acridan compound is preferred as the first chemiluminescent substrate because of the ability to inhibit peroxidase activity (column 7, lines 65-67 to column 8, lines 1-23).

Greene et al. teach inhibitors of the enzyme alkaline phosphatase, which include inorganic phosphate, chelating agents, and amino acids (column 6, lines 9-29), in the context of enzyme-based detection methods using alkaline phosphatase, horseradish peroxidase, and other enzymes (column 6, lines 30-37 and column 10, lines 19-30).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to use a horseradish peroxidase substrate (such as an acridan) in the method of Akhavan-Tafti '979 and Akhavan-Tafti et al. as the second, rather than the substrate, and to use a 1,2-dioxetane substrate capable of being activated by alkaline phosphatase as the first, rather than the second substrate, because Greene et al. teaches that alkaline phosphatase may also be readily inhibited in assays that employ this enzyme, such as those of Akhavan-Tafti '979 and Akhavan-Tafti et al.

51. Claim 43 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cheek et al. in view of Akhavan-Tafti '979 as applied to claims 2, 5, 28, and 44 above, and further in view of Akhavan-Tafti et al. (US Patent No. 5,523,212). Cheek et al. and Akhavan-Tafti '979 are as discussed above, which fails to specifically teach a composition comprising a chemiluminescent quantum yield enhancing material that further comprises counterion moieties listed in claim 43.

Akhavan-Tafti '212 teach chemiluminescent formulations for the detection of biological molecules that comprise enhancers and additives such as β-cyclodextrin, polyols, and sulfate (column 15, lines 45-59; column 16, lines 1-21; and Examples 13 and 20), and it is further taught that useful levels of light intensity compared to reagent

background are obtained with reagents that incorporate dextran sulfate and β-cyclodextrin (Example 20).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ dextran sulfate and β-cyclodextrin, as taught by Akhavan-Tafti '212 in the chemiluminescent formulations comprising enhancers of Akhavan-Tafti '979 and Akhavan-Tafti et al., because Akhavan-Tafti '212 teaches that such additives give rise to useful levels of light intensity in methods for chemilumiminescent detection of biological molecules, such as those of Akhavan-Tafti '979 and Akhavan-Tafti et al.

52. Claim 27 is rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti (US Patent No. 6,068,979) in view of Akhavan-Tafti et al. (*Clinical Chemistry* **44**:2065-2066 (1998)) as applied to claims 1-5, 7, 9, 13-15, 20-21, 28-29, 31-32, 40-41, and 44 above, and as evidenced by Girotti et al. and Akhavan-Tafti (Girotti et al., "Chemiluminescent Immunoperoxidase Assay for the Dot Blot Hybridization Detection of Parvovirus B19 DNA Using a Low Light Imaging Device," *Analytical Biochemistry* 236:290-295 (1996) and US Patent No. 5,650,099).

Use of the chemiluminescent substrates Lumigen PPD and PS-3, which have different emission maxima of 470 nm and 430 nm, respectively, as evidenced by Akhavan-Tafti (US Patent No. 5,650,099, column 22, lines 15-26) and Girotti et al. (p. 290, right column, paragraph 3) is taught by Akhavan-Tafti '979 (column 9, lines 46-47; column 10, lines 42-45, and column 15, lines 1-8).

53. Claim 25 is rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti (US Patent No. 6,068,979) in view of Akhavan-Tafti et al. (*Clinical Chemistry* **44**:2065-2066 (1998)) and as evidenced by Girotti et al. and Akhavan-Tafti as applied to claim 27 above, and further in view of Akhavan-Tafti et al. (US Patent No. 6,045,727).

Akhavan-Tafti et al. (US Patent No. 6,045,727) teach substrates for chemiluminescent detection. In particular, Akhavan-Tafti '727 teaches an Acridan Derivative 1, which has an emission maxima of 430 nm and is capable of being activate by alkaline phosphatase (Examples 1, 14, and 19).

Therefore, it would have been obvious to one of ordinary skill in the art to substitute the Lumigen PPD substrate of Akhavan-Tafti '979 with the Acridan Derivative 1 in the method of Akhavan-Tafti '979 and Akhavan-Tafti et al., because Akhavan-Tafti '727 teaches that Acridan Derivative 1 is a substrate for a hydrolytic enzyme, which is a requirement for the second enzyme in the sequential chemiluminescent detection method of Akhavan-Tafti '979 and Akhavan-Tafti et al.

54. Claims 42-43 are rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti '979 and Akhavan-Tafti et al. as applied to claims 1-5, 7, 9, 13-15, 20-21, 28-29, 31-32, 40-41, and 44 above, and further in view of Akhavan-Tafti (US Patent No. 5,523,212).

Akhavan-Tafti '979 and Akhavan-Tafti et al. teach preferred formulations for chemiluminescence but fails to specifically teach additives and counterions. Akhavan-Tafti '212 teach chemiluminescent formulations for the detection of biological molecules

that comprise enhancers and additives such as β-cyclodextrin, polyols, and sulfate (column 15, lines 45-59; column 16, lines 1-21; and Examples 13 and 20), and it is further taught that useful levels of light intensity compared to reagent background are obtained with reagents that incorporate dextran sulfate and β-cyclodextrin (Example 20).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ dextran sulfate and β-cyclodextrin, as taught by Akhavan-Tafti '212 in the chemiluminescent formulations comprising enhancers of Akhavan-Tafti '979 and Akhavan-Tafti et al., because Akhavan-Tafti '212 teaches that such additives give rise to useful levels of light intensity in methods for chemilumiminescent detection of biological molecules, such as those of Akhavan-Tafti '979 and Akhavan-Tafti et al.

55. Claims 6 and 33-34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti '979 and Akhavan-Tafti et al. as applied to claims 1-5, 7, 9, 13-15, 20-21, 28-29, 31-32, 40-41, and 44 above, and further in view of Bronstein et al. (US Patent No. 4,931,223). Akhavan-Tafti '979 and Akhavan-Tafti et al. are as discussed above, which fail to specifically teach a method wherein both the first and second chemiluminescent substrates are 1,2-dioxetane substrates or a method where the enzymes are β-galactosidase and alkaline phosphatase.

Bronstein et al. teach methods for detecting chemiluminescent emissions using two or more 1,2-dioxetane substrates, which may be used in quantifying several analytes when each of the 1,2-dioxetanes emits light of a different wavelength (column

2, line 42 to column 3, line 3; 6, lines 44-47; column 7, lines 5-19; column 8, lines 1-29 in particular). Bronstein et al. further teach that β-galactosidase and alkaline phosphatase may be used in the method for cleaving different cleavable dioxetane substituents, and that use of these enzymes to cleave 1,2-dioxetane substrates that emit light of different wavelengths enables multichannel assays to be performed (column 7, lines 5-19; column 13, "Assay Procedure").

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ two 1,2-dioxetane substrates in the method of Akhavan-Tafti '979 and Akhavan-Tafti et al. because Bronstein et al. teach that two or more 1,2-dioxetane substrates may be successfully used in a chemiluminescent detection assay for the quantification of two or more analytes, such as that of Akhavan-Tafti and Akhavan-Tafti et al., in order to enable multichannel assays.

56. Claim 8 is rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti '979 and Akhavan-Tafti et al. as applied to claims 1-5, 7, 9, 13-15, 20-21, 28-29, 31-32, 40-41, and 44 above, and further in view of Bronstein et al. (US Patent No. 6,602,658 B1).

Akhavan-Tafti '979 and Akhavan-Tafti et al. fail to teach a method wherein the surface layer is contacted with the enhancing material prior to contacting with the first chemiluminescent substrate composition.

Bronstein et al. teach a method of measuring gene activity using sequential chemiluminescent detection of signal from two or more chemiluminescent substrates, wherein chemiluminescent signal enhancers such as onium copolymers,

polyvinylbenzyltrimethylammonium chloride or BSA may be added to increase the intensity of the chemiluminescent signals in aqueous medium (column 11, lines 29-61). Bronstein et al. further teach that the enhancer molecule can be added at any point during the method (column 13, lines 39-52).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to provide the quantum yield enhancing material before contacting the surface layer with the first chemiluminescent substrate composition, because Bronstein et al. teach that the enhancing material may be added at any point in a method of sequential detection of chemiluminescent signals such as that of Akhavan-Tafti '979.

57. Claim 24 is rejected under 35 U.S.C. 103(a) as being unpatentable over Akhavan-Tafti '979 and Akhavan-Tafti et al. as applied to claims 1-5, 7, 9, 13-15, 20-21, 28-29, 31-32, 40-41, and 44 above, and further in view of Akhavan-Tafti et al. (US Patent 5,843,666). Akhavan-Tafti '979 and Akhavan-Tafti et al. are as discussed above, which fail to teach a chemiluminescent detection method in which the support surface further comprises fluorescent labels.

Akhavan-Tafti '666 teaches chemiluminescent detection methods for detection of multiple DNA sequences, wherein DNA is immobilized on a solid support (nylon membrane). The DNA comprises a fluorescent label (fluorescein), which enables chemiluminescent detection following recognition by an anti-fluorescein antibody-enzyme conjugate (HRP-anti-fluorescein) (columns 12-13, Example 1).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to include the fluorescent label of Akhavan-Tafti '666 in the method of Akhavan-Tafti and Akhavan-Tafti et al. for the purpose of enabling recognition by an anti-fluorescein antibody-enzyme conjugate and allowing for signal detection in a method of chemiluminescent detection.

Double Patenting

58. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

59. Claims 1-45 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-45 of copending Application No. 10/620,333 (US Patent Application Publication 2005/0026151 A1 in view of Akhavan-Tafti et al. (US Patent No. 6,068,979).

Although the conflicting claims are not identical, copending Application No. 10/620,333 also claims a method of detecting chemiluminescent emissions on a solid support, the method comprising: contacting a surface layer of the solid support with a composition comprising first and second chemiluminescent substrates capable of being activated by first and second enzymes to produce a first and second chemiluminescent signals; and detecting the first and second chemiluminescent signals on the surface layer of the solid support; wherein a plurality of probes are disposed in a plurality of discrete areas on the surface layer at a density of at least 50 discrete areas per cm^2, wherein at least some of the probes are bound to a first enzyme conjugate comprising the first enzyme, and wherein at least some of the probes are bound to a second enzyme conjugate comprising the second enzyme. Application No. 10/620,333 fails to specifically teach sequential detection of the first and second chemiluminescent signals.

However, Akhavan-Tafti et al. teach a method of detecting chemiluminescent emissions on a solid support using two chemiluminescent substrates and two enzymes capable of activating the substrates, wherein the first and second chemiluminescent signals are detected sequentially (see column 7, lines 24-25 and 35-46 in particular). Therefore, it would have been obvious to one of ordinary skill in the art to include two chemiluminescent substrates and two enzymes capable of activating the substrates, as taught by Akhavan-Tafti '979, in order to detect multiple chemiluminescent emissions/multiple target species. This is a provisional obviousness-type double patenting rejection.

60. Claims 1-45 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 28-57 of copending Application No. 10/462,742 (US Patent Application Publication 2004/0259182 A1 in view of Akhavan-Tafti et al. (US Patent No. 6,068,979).

Although the conflicting claims are not identical, copending Application No. 10/462,742 also claims a method of detecting chemiluminescent emissions on a solid support, the method comprising: contacting a surface layer of the solid support with a composition comprising a chemiluminescent substrate capable of being cleaved by an enzyme to produce chemiluminescence; and detecting chemiluminescent emissions from the surface layer of the solid support; wherein a plurality of probes are disposed in a plurality of discrete areas on the surface layer, wherein the density of discrete areas on the surface layer is at least 50 discrete areas per cm^2, wherein at least some of the probes are bound to an enzyme conjugate comprising an enzyme capable of cleaving the chemiluminescent substrate, and wherein the composition comprising the chemiluminescent substrate is contacted with the surface layer in the presence of a chemiluminescent enhancing material. Application No. 10/462,742 fails to specifically teach sequential detection of multiple second chemiluminescent signals.

However, Akhavan-Tafti et al. teach a method of detecting chemiluminescent emissions on a solid support using two chemiluminescent substrates and two enzymes capable of activating the substrates, as discussed above. Therefore, it would have been obvious to one of ordinary skill in the art to include two chemiluminescent substrates and two enzymes capable of activating the substrates, as taught by Akhavan-Tafti '979,

in order to detect multiple chemiluminescent emissions/multiple target species. This is a provisional obviousness-type double patenting rejection.

Conclusion

61. No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christine Foster whose telephone number is (571) 272-8786. The examiner can normally be reached on M-F 8:30-5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long Le can be reached at (571) 272-0823. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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